Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigations of **Polysorbate 60** in the developing chicken embryo 12/29/77

E24

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: December 29, 1977

Through: HFF-150 _____

FROM : Supervisory Chemist
Whole Animal Toxicology Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the in-house investigations of Polysorbate 60 in the developing chicken embryo.

Jacqueline Verrett

Jacqueline Verrett, Ph.D.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Polysorbate 60

Protocol:

Polysorbate 60 (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in ethanol as the solvent by two routes and two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square Test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Polysorbate 60 showed little toxicity under the test conditions employed. Air cell treatment at 0 hours resulted in a calculated LD_{50} of 1567.55 mg/kg (78.38 mg/egg), while yolk treatment at 0 hours gave an LD_{50} of 350.93 mg/kg (17.54 mg/egg). No LD_{50} could be calculated for the other two test conditions.

There were a few scattered abnormalities observed throughout the four test conditions, but the anomalies were not different from nor significantly higher in incidence than those observed in the background. Polysorbate 60 displayed no teratogenicity under the test conditions employed.

1. Polysorbate 60, ICI America Inc., Wilmington, Delaware, Lot #1289

2. McLaughlin, J., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol 5, 760-770

3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOC 47, 1002-1006

4. Finney D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Polysorbate 60 Air Cell at 0 Hours

Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.00	500.00	104	53.84*	4.80	2.88
12.50	250.00	105	44.76*	4.76	1.90
5.00	100.00	105	31.42	1.90	1.90
2.50	50.00	105	38.09	2.85	0.95
1.250	25.00	105	29.52	0.95	0.00
Ethanol		105	30.47	0.95	0.00
Controls		301	14.28	0.00	0.00

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 1567.55 mg/kg (78.38 mg/egg)

Polysorbate 60 Air Cell at 96 Hours Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
1.00	20.00	105	50.47	3.80	2.85
0.50	10.00	105	60.95*	2.85	0.00
0.20	4.00	105	45.7	0.95	0.00
0.10	2.00	105	42.85	3.00	1.90
0.050	1.00	105	53.33	2.85	0.95
Ethanol		110	44.54	2.72	2.72
Controls		301	14.28	0.00	0.00

*Significantly different from solvent $p \leq 0.05$

**Slope is negative

Polysorbate 60
Yolk at 0 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.00	500.00	105	70.47*	0.95	0.00
12.50	250.00	105	54.28*	0.95	0.95
5.00	100.00	105	57.14*	1.90	0.00
2.50	50.00	105	48.57*	0.00	0.00
1.250	25.00	105	40.00	0.95	0.00
Ethanol		105	29.52	0.95	0.00
Controls		301	14.28	0.00	0.00

*Significantly differnet from solvent $p \leq 0.05$

**LD_{50} 350.93 mg/kg (17.54 mg/egg)

Polysorbate 60 Yolk at 96 Hours

Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
1.00	20.00	105	38.09	0.00	0.00
0.50	10.00	105	42.85*	2.85	1.90
0.20	4.00	105	38.09	0.00	0.00
0.10	2.00	105	41.90*	3.80	3.80
0.050	1.00	105	33.33	0.00	0.00
Ethanol		110	25.45	0.00	0.00
Controls		301	14.28	0.00	0.00

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$